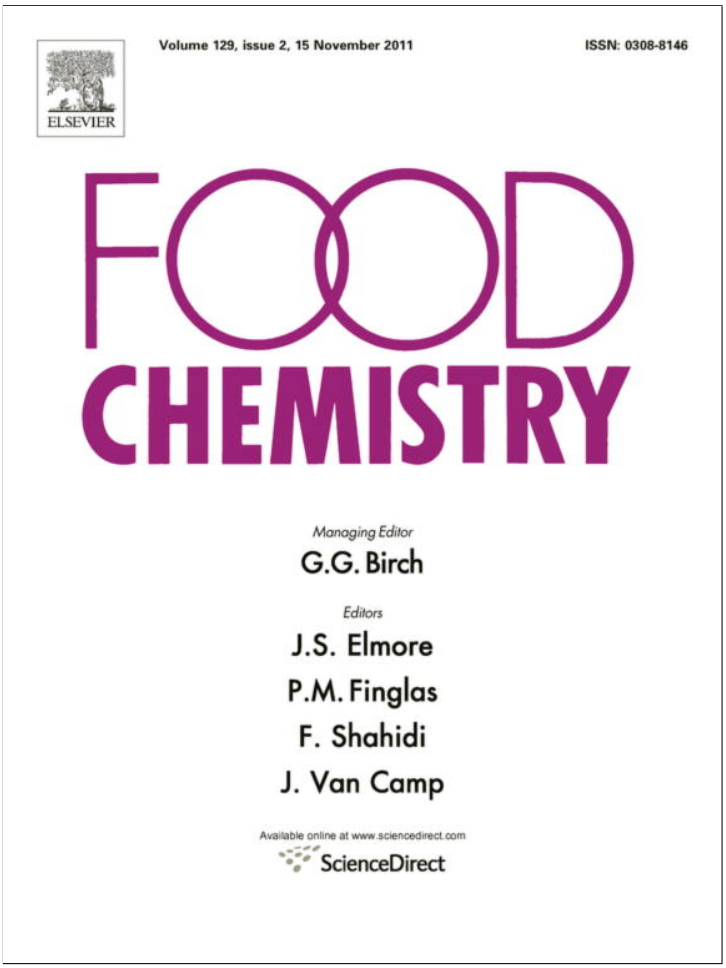

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Analytical Methods

Folate composition of 10 types of mushrooms determined by liquid chromatography–mass spectrometry

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ABSTRACT

White button, crimini, shiitake, maitake, enoki, oyster, chanterelle, morel, portabella, and uv-treated portabella mushrooms were sampled from U.S. retail outlets and major producers. Folate [5-methyltetrahydrofolate (5-CH_3-H_4folate), 10-formyl folate (10-HCO-folate), 5-formyltetrahydrofolate (5-HCO-H_4folate)] was analysed using a validated LC–MS method in four composites of each product, including an in-house mushroom control composite and a reference material (BCR 485 Lyophilised Mixed Vegetables). Chanterelle and morel had the lowest total folate (2–6 μg/100 g), oyster had the highest (mean, 44.2 μg/100 g); other types contained 12.4 μg/100 g (shiitake) to 29.8 μg/100 g (vitamin D-enhanced portabella). Enoki and oyster had almost exclusively 5-CH_3-H_4folate. Morel and chanterelle contained predominately formyl folates. Other species had similar amounts of 5-CH_3-H_4folate and formyl folates. Enoki, oyster, and shiitake, unlike all others, had low to non-detectable 10-HCO-folate (<1 μg/100 g). These precise data on the composition of folate vitamers in different types of mushrooms will facilitate assessment of the dietary contribution of naturally occurring folate.

1. Introduction

The importance of folate in foetal neural tube development has long been recognised and well reviewed, along with more recent research on the role of folate in reducing the risk of other disorders including cardiovascular disease, thromboembolitic processes, colon cancer, and neuropsychiatric disorders (Gilbody, Lewis, & Lightfoot, 2007; Kim, 2003; Lucock, 2000; Muskiet & Kemperman, 2006; Regland, 2005; Stover, 2004). Routine enrichment of refined cereals and grains and infant formulas began in 1998 (U.S. Food, 1996). Although these products remain a primary source of folate in the U.S. and other developed countries, endogenous food folate is the sole source of the vitamin in the absence of enriched foods or dietary supplements, for example in underdeveloped countries or in very low carbohydrate diets (Last & Wilson, 2006). Among plant foods, whole grains, dark leafy greens, and citrus fruits are recognised as good sources of folate. Recent research efforts have been directed at genetic modification of staple food crops to increase endogenous folate (Storozhenko et al., 2005).

Folic acid (pteroylglutamic acid) is used for enrichment, but very little folic acid occurs naturally in foods. Aside from liver,

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the most common sources of endogenous food folate are fruits, vegetables, and whole grains, and the major vitamers are 5-methyltetrahydrofolate (5-CH_3-H_4folate), 5-formyltetrahydrofolate (5-HCO-H_4folate), 10-formylfolate (10-HCO-folate), 10-formyldihydrofolate (10-HCO-H_2folate), and tetrahydrofolate (H_4folate), which occur as polyglutamates (Konings et al., 2001). 5-CH_3-H_4folate is the predominant folate in most fruits and vegetables, while whole grains are richer in formyl folates (Finglas et al., 1999; Kariluoto, Vahteristo, & Piironen, 2001; Konings et al., 2001).

5-CH_3-H_4folate functions as the methyl donor in conversion of homocysteine to S-adenosylmethionine, a key source of methyl groups in single-carbon metabolism including DNA methylation and brain chemistry (Gilbody et al., 2007; Stover, 2004). Dietary folic acid is converted to 5-CH_3-H_4folate, and the enzyme methylenetetrahydrofolate reductase (MTHFR) is key in this process. Certain MTHFR polymorphisms result in decreased MTHFR activity and have been correlated with increased incidence of mental disorders (Gilbody et al., 2007), certain types of cancer, and other diseases (Robien & Ulrich, 2003; Sharp & Little, 2004). 5-CH_3-H_4folate has also demonstrated free radical scavenging activity (Moat et al., 2004). Consequently, naturally occurring folate may be especially important in individuals with MTHFR mutations, the incidence of which varies by ethnicity and geographic population (Gilbody et al., 2007), and naturally occurring food folate has been shown to increase plasma folate concentrations similar to folic acid fortified foods (Ashfield-Watt et al., 2003).

Accurate food composition data are essential for research that requires estimation of folate intake, and also for making dietary recommendations. Quinlivan, Hanson, and Gregory (2006) have reviewed the occurrence of folates and their determination in biological matrices. The current standard methodology by which most food composition data for folate have been generated is a microbiological assay, which measures total folate based on the growth response of specific bacteria after extraction from the food matrix using amylase and protease, followed by deconjugase to hydrolyse polyglutamyl folates to monoglutamates (Angyal, 1996; DeVries, Keagy, Hudson, & Rader, 2001; DeVries et al., 2005). This methodology is generally known as tri-enzyme extraction, although some laboratories will omit the amylase and protease for samples low in starch and protein, respectively. The microbiological assay has been shown to yield variable and often inaccurate results (Konings, 2006; Koontz et al., 2005; Puwastien, Pinprapaia, Judprasonga, & Tamura, 2005), especially for naturally occurring folate in many food matrices (Koontz et al., 2005). Additionally this method does not distinguish different vitamers. Alternatively, chemically specific determination of food folate has been accomplished using high performance liquid chromatography with either fluorescence detection (Konings, 1999) or by liquid chromatography–mass spectrometry (LC–MS) with quantification using stable isotope dilution (Freisleben, Schieberle, & Rychlik, 2003; Rychlik, 2004; Rychlik, Englert, Kapfer, & Kirchhoff, 2007) or external standards (Patring, Wandel, Jägerstad, & Frølich, 2009; Phillips, Ruggio, Ashraf-Khorassani, & Haytowitz, 2006). Food composition data from this methodology have been reported for a number of foods, but there is scant information on folate in various mushrooms, as noted in recent reviews on mushroom composition (Cağlarırmak, 2007; Kalač, 2009).

Current average food composition data indicate mushrooms are a good source of folate. The total folate content of several types of mushrooms reported in the United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference (SR) in 2009 ranged from 14–52 μg/100 g, based on microbiological analysis (USDA Agricultural Research Service, 2009). Mattila and coworkers (Mattila et al., 2001) reported total folate of 25–55 μg/100 g in a few types of mushrooms sampled in Sweden. Yon and Hyun (2003) reported total folate of 56–80 μg/100 g in three types of mushrooms in Korea (oyster, pyogo, and top). All of these data are based on the standard microbiological assay.

As part of the USDA's National Food and Nutrient Analysis Program (NFNAP) (Haytowitz, Pehrsson, & Holden, 2007), 10 types of mushrooms available in retail markets in the U.S. were sampled nationwide for analysis of a range of nutrients to update food composition data in SR (USDA Agricultural Research Service, 2009). Folate was analysed using a previously validated LC–MS method (Phillips, Ruggio et al., 2006), and results of these analyses are reported.

2. Materials and methods

2.1. Samples

Samples were procured in April 2009 according to a plan designed to complement an earlier sampling in November 2004, by including mushrooms exposed to uv light to increase the vitamin D content, as well as other types not previously sampled (morel and chanterelle). White button, portabella, vitamin D-enhanced portabella (uv treated), maitake, enoki, shiitake, oyster, and crimini mushrooms were sampled from retail markets in 12 U.S. cities, according to a statistically representative plan developed for the NFNAP (Pehrsson, Haytowitz, Holden, Perry, & Beckler, 2000). In a few cases (shiitake, oyster, and crimini) where samples could not be found in all retail locations, samples were either supplemented with additional samples from producers or local samples (Blacksburg, VA) were obtained. Samples (0.6–3 kg each) of vitamin D-enhanced portabella (uv treated), enoki, maitake, chanterelle, and morel mushrooms were obtained from at least two producers. For the samples procured from retail outlets, approximately 200–1000 g per mushroom type (either loose or packaged) was purchased from each market. The mushroom samples were packaged in perforated coolers containing freezer packs, taking care to avoid contact between the samples and the freezer packs, and shipped via overnight service to the Food Analysis Laboratory at Virginia Tech (Blacksburg, VA). Upon receipt, the product label and visual appearance were used to verify sample identification. All samples were refrigerated (2–8 °C) between receipt and compositing/homogenisation, with storage time ranging from <1 day to 6 days.

Samples of mushrooms obtained from retail outlets were combined to create four composites, each comprising samples from three randomly selected locations. Each composite comprised a subsample of approximately 200–500 g per retail outlet, with approximately equal weight (±50 g) from each outlet and no subsampling of individual mushrooms. For samples shipped directly from suppliers, approximately 0.5–2.3 kg were used for each composite, depending on the amount of product provided. Samples from different producers were not combined, although separate lots from each producer were composited individually.

Preparation and homogenisation of each composite was performed in a uv-protected environment. At the time of compositing, mushrooms were removed from their packaging, placed on a lint-free cloth, and gently brushed with a kitchen brush and wiped with a lint-free cloth dampened with distiled, deionised water to remove excess debris. The mushrooms were then placed on a cutting board and trimmed of inedible/damaged portions and the base of the stems using a stainless steel knife. For portabella mushrooms only, the entire stem was removed and discarded. After cleaning and trimming, the mushrooms were cut into pieces of ~1.25 cm, frozen in liquid nitrogen, then homogenised using a 6L stainless steel industrial food processor (Robot Coupe Blixer®, Robot Coupe USA, Jackson, MS) while being kept frozen with liquid nitrogen. Subsamples (10–15 g) of the frozen composite were dispensed into

60-mL glass jars with Teflon®-lined lids, surrounded with aluminium foil, and stored in darkness at –60 °C prior to analysis, which took place within 10–16 weeks. Stability of folate under these storage conditions had been established (Phillips et al., 2005).

A control composite (Mushroom CC) for use as an analytical quality control material was also prepared, and comprised 2.46 kg total of approximately equal weights of locally purchased portabella mushrooms and vitamin D-enhanced portabella mushrooms (1.25 kg vitamin D- enhanced, 1.21 kg non-enhanced) obtained directly from the supplier (Dole, West Chester, PA). The mushrooms were cleaned, trimmed, and homogenised as described above, except they were processed in two batches using two separate food processors, each containing approximately half of each type of mushroom. The homogenates were poured alternately from the two processors into a large bowl, additional liquid nitrogen was added, the composite was stirred for 2 min using a stainless steel spoon, and then while the composite was maintained frozen with liquid nitrogen it was distributed among 192 60-mL glass jars with Teflon®-lined closures, with stirring to maintain homogeneity during dispensing. Subsamples were dispensed and stored as described above.

Moisture in each composite, and in aliquots taken from throughout the dispensing sequence of the control composite, was determined by vacuum drying to constant weight at 65–70 °C and 635 mm Hg. These data were used to verify the homogeneity of the control composite prior to its use and to obtain the dry mass of each mushroom sample composite.

2.2. Analysis of folate

Folate was assayed by LC–MS after tri-enzyme extraction using methodology previously reported (Phillips, Ruggio et al., 2006; Phillips et al., 2005), except only 5-CH_3-H_4folate, 10-HCO-folate, and 5-HCO-H_4folate were measured in all composites. One composite of each type of mushroom was screened for presence of 10-CH_3-folate, 5,10-methenyltetradydrofolate (5,10-CH^+-H_4folate), 5-methyldihydrofolate (5-CH_3-H_2folate), and 5,10-methylene-tetrahydrofolic acid (5,10-CH_2-H_4folate). None of the samples showed any of these components, with a limit of detection of 0.3–0.4 μg/100 g based on the instrument calibration range, although the stability of 5,10-CH_2-H_4folate and 5-CH_3-H_2folate was questionable based on poor, absent, or variable response of the standards.

Total folate as dietary folate equivalents was calculated as the sum of the molar concentration of each folate multiplied by the molecular mass of folic acid.

2.3. Quality control

The homogeneity of the Mushroom CC was evaluated and determined to be acceptable using a previously described approach (Phillips et al., 2006). Briefly, moisture was assayed in triplicate in each of seven subsamples drawn from across the dispensing sequence of the total of 176 subsamples and an analysis of variance among the subsamples was performed. Also the magnitude of the overall range and relative standard deviation were evaluated.

A subsample of the Mushroom CC was included in each assay batch, and the commercial reference material BCR485 Lyophilised Mixed Vegetables (Institute for Reference Materials and Measurements, Geel, Belgium), which is provided with an indicative value for 5-methyltetrahydrofolate, was assayed in two of the total of five analytical batches. The four composites of each type of mushroom were assayed in different analytical batches to incorporate inter-assay analytical uncertainty. Additionally, each assay batch included at least one composite assayed in duplicate as an inter-assay quality control check.

The limit of detection (LOD) and limit of quantification (LOQ) in mushroom samples were 0.4 and 2 μg/100 g fresh weight, respectively (except 50% lower for 10-HCO-folate because of greater sensitivity), and were estimated based on the observed signal to noise ratio (S/N) for the analytes in mushroom samples, the instrumental limits of detection and calibration range, and the sample size (2 g) and dilutions used in extraction and preparation of samples for LC–MS analysis. The S/N in mushroom sample extracts with the analyte peak at an intensity similar to the lowest standard of the external calibration curve in each case was evaluated. For all components, the S/N for the lowest standard was 50–100, which yielded a response well above that necessary for accurate quantification. The estimated LOD in samples with an analyte concentration of 0.4 μg/100 g (0.2 μg/100 g for 10-HCO-folate) had a S/N of 10–20, which was well above baseline noise and easily distinguishable as a definite peak response.

2.4. Data analysis

Means and standard deviations were calculated using Microsoft® Excel (Professional edition 2007; Microsoft Corporation, Redmond, WA). Analysis of variance ($\alpha = 0.05$) was performed using Quattro Pro© (version 14.0.0.603, Corel Corporation; Ottawa, Ontario). For the Mushroom CC, the expected ratio of the actual relative standard deviation (RSD) to the expected RSD (HORRAT) for replicates was calculated as described by Horwitz and coworkers (Horwitz, Kamps, & Boyer, 1980).

Results for the lyophilised reference material were calculated on a dry mass basis for comparison to the values in the certificate of analysis (COA), using moisture determined as specified in the COA (Finglas, Scott, Witthöft, van den Berg, & de Froidmont-Görtz, 1998).

3. Results

3.1. Quality control

The mean assayed moisture content of the Mushroom CC was 91.57 g/100 g, with 0.09% RSD and a range of 91.44–91.69 g/100 g. The homogeneity of the composite was supported by the lack of statistically significant difference ($p < 0.5$) between subsamples, in the context of an established inter-assay precision of 0.14% for an in-house mixed food control material having a similar moisture content.

Table 1 summarises the folate results for the Mushroom CC and BCR485 Lyophilised Mixed Vegetables reference material. For the Mushroom CC, the RSDs for 5-CH_3-H_4folate, 10-HCO-folate, 5-HCO-H_4folate, and the sum of these vitamers for the controls assayed with the mushroom samples demonstrated good analytical precision and allowed confident comparison of folate concentrations among composites at concentrations above the LOQ of 2 μg/100 g. The precision was similar for BCR485, although the concentrations of 5-CH_3-H_4folate and sum of assayed folates in this lyophilised material were much higher than in the Mushroom CC, due to the low moisture content and different composition. 5-CH_3-H_4folate was within the indicative (non-certified) range for the reference material.

The assayed total folate concentration of BCR485 as the sum of the individual vitamers summed as molar equivalents of folic acid was lower than the certified total folate concentration that was determined by microbiological assay (Finglas et al., 1998). This result is consistent with the known discrepancy that can occur between microbiological versus chromatographic analysis (Koontz et al., 2005; Phillips et al., 2010; Puwastien et al., 2005) and also addressed by Konings (2006). It is interesting that the total formyl

Table 1
Quality control results for folate (μg/100 g) in the Mushroom control composite and in BCR 485 Lyophilised Mixed Vegetables reference material (Institute of Reference Materials and Methods, Geel, Belgium).

Control material	Parameter[a]	Component[b] (μg/100 g fresh weight)			
		5-CH_3-H_4folate	10-HCO-folate	5-HCO-H_4folate	Sum of folates
Mushroom control composite:					
Assayed with mushroom samples[c]	Mean	24.5	4.51	3.32	30.9
	SD	1.2	0.35	0.19	0.68
	%RSD	4.8	7.7	5.7	2.2
	n	3	3	3	3
Overall within-laboratory[d]	Mean	24.1	4.55	3.30	30.7
	SD	1.7	0.64	0.14	1.62
	%RSD	6.9	14.0	4.3	5.3
	n	5	6	6	5
	HORRAT[e]	0.5	0.6	0.4	0.2
BCR485 Lyophilised Mixed Vegetables:					
Assayed with mushroom samples[c]	Low	208	<1.0	3.24	204
	High	245		4.63	240
	n	2	2	2	2
Overall within-laboratory[f]	Mean	238	<1.0	3.49	237
	SD	15.3	–	0.47	16.8
	%RSD	6.4	–	13.5	7.1
	Mean+/−2SD	207–268	–	2.55–4.43	203–271
	n	37	8	8	8
	HORRAT[e]	1.0	–	1.0	0.8
Certificate of analysis[g]	Mean ± 2SD	172–256	n/a	n/a	287–343

[a] SD = standard deviation; RSD = relative standard deviation.
[b] 5-CH_3-H_4folate = 5-methyltetrahydrofolate; 10-HCO-folate = 10-formylfolate; 5-HCO-H_4folate = 5-formyltetrahydrofolate; sum of folates = sum of molar concentration of individual vitamers, as molar equivalent mass folic acid.
[c] Assayed in separate analytical batches.
[d] Data from all assays over a period of 1 year.
[e] Assayed RSD divided by expected RSD (Horwitz et al.,1980).
[f] All assays over a period of 6 years (5-CH_3-H_4folate) and 4 years (10-HCO-folate and 5-HCO-H_4folate).
[g] Certified value for total folate by microbiological assay; indicative value for 5-CH_3 -H_4folate determined by HPLC (Finglas et al., 1998).

folate content of the Mushroom CC on a dry mass basis (93 μg/100 g) was more than 26 times the concentration of total formyl folates in BCR485 (3.49 μg/100 g), which is a lyophilised mixture of canned tomatoes, frozen carrots, and sweet corn.

3.2. Folate content and composition of mushrooms

Table 2 summarises the sum of assayed vitamers as folate equivalent concentrations in the 10 types of mushrooms. All results are given on a fresh weight basis. The moisture contents (Table 2) did not differ by a large amount among composites.

The average folate concentration in chanterelle and morel mushrooms (2.22 and 6.40 μg/100 g, respectively) was quite low relative to other species, which had average folate contents ranging from 12.4 μg/100 g (shiitake) to 44.2 μg/100 g (oyster). Oyster and enoki mushrooms had the highest mean folate content (44.2 and 42.5 μg/100 g, respectively) by a large margin, although there were notable differences among individual composites of these varieties. In fact, the concentration in the oyster and enoki composites with the lowest folate content (30.2 and 20.7 μg/100 g, respectively) was similar to the concentration in all other mushrooms (12.4–29.8 μg/100 g) besides chanterelle and morel, and approximately half the concentration in the enoki and oyster composites that had the highest folate (61.4 and 67.8 μg/100 g, respectively). There was no statistically significant difference in folate in the uv-treated and non-treated portabella mushrooms. The highly consumed white button and portabella mushrooms had the narrowest difference (2.83–6.01 μg/100 g) in folate content among separate composites.

The vitamer composition differed among different species, and even within composites of a particular mushroom type in some cases, as illustrated in Fig. 1. 5-CH_3-H_4folate was by far the major individual vitamer in the mushrooms with the highest total folate content (enoki and oyster), representing 60–90% of the sum of assayed folates (Fig. 1). Interestingly, these two species also were the only ones containing no detectable 10-HCO-folate (<1 μg/100 g) in all composites; shiitake mushrooms, while having a much lower sum of folates, also contained very little 10-HCO-folate. In other varieties there were not widely disparate concentrations of 5-CH_3-H_4folate and total formyl folates, except the morel and chanterelle mushrooms (which had a low sum of folates) contained predominately formyl folates (76–88% of the total). Some species showed variability among composites. In shiitake mushrooms, two of the four composites had mostly formyl folates, but 5-CH_3-H_4folate predominated in the other two. In crimini mushrooms, total formyl folates comprised approximately 30% of total folate in all four composites, but 5-HCO-H_4folate predominated in two and 10-HCO-folate was the major formyl folate in the others. It is interesting that there appeared to be a slightly higher proportion of 5-HCO-H_4folate in the uv-treated portabella samples compared to the untreated portabellas (Fig. 1), although definitive conclusions cannot not be drawn since the experimental design was not a controlled study of uv exposure.

Quinlivan et al. (2006) have reviewed folate synthesis and metabolism, and Cossins and Liangfu (1997) have reviewed folate metabolism in plants and fungi. The differences in folate composition observed in certain types of mushrooms (Fig. 1) might reflect variability in biochemical pathways among different genera or species, or differences in growing or processing conditions.

4. Discussion

Relatively little data exist in the literature on the folate content or composition of mushrooms. Most studies on mushroom composition have focused on other nutrients and components. In Fig. 2,

Table 2
Folate composition of individual mushroom composites and average total folate content for 10 types of mushrooms. Composites having a letter code came directly from a supplier; numbered composites represent retail sample composites, as described in the text (*Samples*).

Description	Scientific name	NDB number[a]	Composite	Moisture (g/100 g)	Component[b] (μg/100 g fresh wt.)						
					5-CH_3-H_4folate	10-HCO-folate	5-HCO-H_4folate	Sum of folates[c]			
								Assayed	n	Mean	StdErr[d]
Portabella, vitamin D enhanced	*Agaricus bisporus*	11998	A1	94.86	16.1	3.50	6.24	24.6	4	29.8	3.75
			A2	95.12	15.0	3.61	5.38	22.8			
			B1	94.76	27.5	3.85	9.53	39.0			
			B2	93.68	18.7	5.17	10.6	32.7			
White button	*Agaricus bisporus*	11260	1	92.85	9.12	4.28	3.82	16.4	4	17.6	0.59
			2	92.87	8.46	3.00	7.07	17.6			
			3	92.35	8.20	3.14	6.90	17.3			
			4	92.47	11.8	2.46	5.94	19.2			
Enoki	Flammulina veluptipes	11950	A1	87.68	59.7	<1	4.09	61.4	3	42.5	10.9
			A2	88.47	27.7	<1	3.71	30.2			
			B1	88.30	30.5	<1	7.14	35.9			
Shiitake	Lentinus edodes	11238	1	90.53	3.57	<1	12.9	16.1	4	12.4	1.42
			2	86.90	6.00	<1	3.59	9.30			
			3	91.41	1.54	3.19	7.61	11.6			
			A1	90.11	6.92	<1	5.74	12.5			
Maitake	Grifola frondosa	11993	A1	88.37	4.82	3.60	7.55	15.1	3	15.5	1.10
			A2	88.54	7.10	3.21	4.25	13.8			
			B1	92.30	9.39	2.84	6.27	17.6			
Oyster	Pleurotus ostreatus	11987	A1	89.70	66.0	<1	4.59	67.8	3	44.2	13.7
			1	88.65	40.1	<1	5.86	44.0			
			2	90.54	17.7	<1	3.61	20.5			
			2	90.54	18.1	<1	3.42	20.8			
Crimini	Agaricus bisporus	11266	1	91.97	16.1	9.30	<1.00	24.9	4	26.3	5.98
			2	91.22	14.0	4.92	<1.00	18.6			
			A1	93.08	12.4	1.76	4.88	18.1			
			B1	92.07	35.3	1.24	9.19	43.7			
Portabella	Agaricus bisporus	11265	1	90.96	15.2	7.40	5.81	27.0	4	24.5	1.61
			2	92.22	12.5	7.28	2.82	21.5			
			3	91.30	12.4	8.21	2.37	21.9			
			4	91.25	18.8	5.13	4.95	27.5			
Chanterelle	Cantharellus californicus or C. cibarius	11239	A1	91.09	<2	<1	<2	2.46	2	2.22	0.24
			B1	88.61	<2	<1	<2	1.98			
Morel	*Morchella spp.*	11240	A1	89.46	<2	1.99	5.15	7.62	4	6.40	0.63
			A2	90.38	<2	1.83	4.34	7.10			
			B1	89.44	<2	2.19	1.67	4.77			
			B2	89.18	<2	2.96	2.03	6.12			

[a] USDA Nutrient Database number (USDA Agricultural Research Service, 2009).
[b] 5-CH_3-H_4folate = 5-methyltetrahydrofolate 10-HCO-folate = 10-formylfolate; 5-HCO-H_4folate = 5-formyltetrahydrofolate.
[c] sum of individual folates as molar equivalent μg folic acid/100 g.
[d] Standard error for the mushroom type based on $n = 4$ composites. The estimated within-composite uncertainty can be estimated based on the results for the control composite (Table 1).

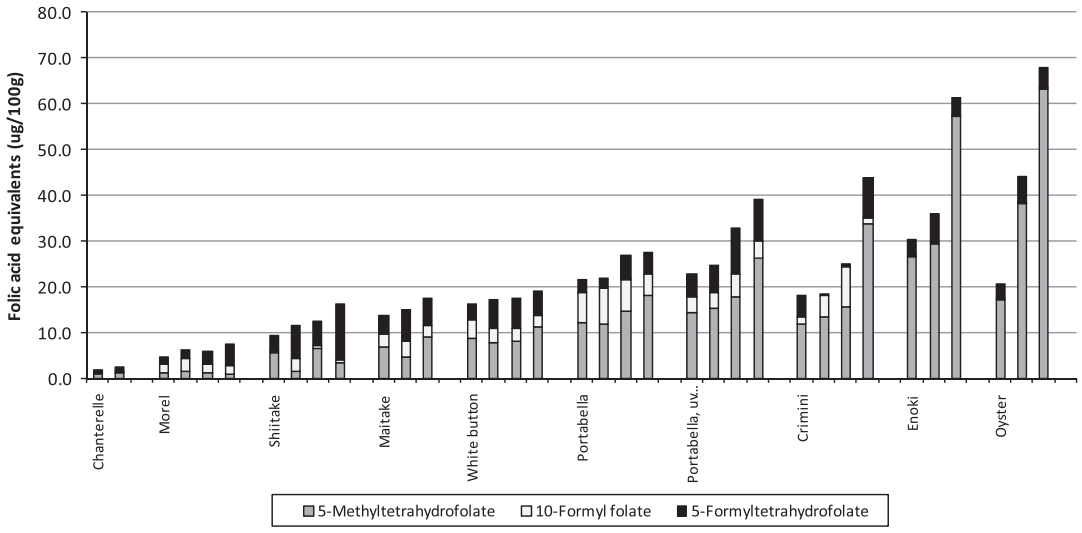

Fig. 1. Folate composition of individual mushroom composites. Composites having a letter code came directly from a supplier; numbered composites represent retail sample composites, as described in the text (*Samples*).

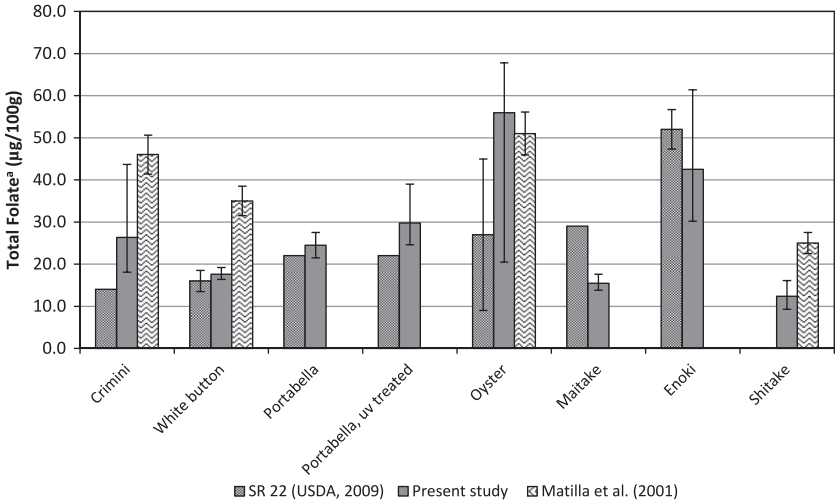

Fig. 2. Comparison of the total folate content of nationwide samples of 10 types of mushrooms in the present study to 2009 USDA Nutrient Database values (SR 22) (USDA Agricultural Research Service, 2009), and to concentrations in selected types reported by Mattila et al. (2001). All data besides those from the present study were generated by microbiological assay. Error bars indicate the low and high folate concentrations in the four composites of each type of mushroom, two times the standard error for SR22 values, and 10% as the estimated uncertainty reported by Mattila et al. (2001). Where no values are shown for a given data set, no data were available for that product.

the folate values in SR in 2009 (USDA Agricultural Research Service, 2009), which were determined by microbiological analysis, are compared to the concentrations measured in the current study and to results reported by Mattila et al. (2001) for some of the types. There were no data in SR in 2009 for morel, chanterelle, or shiitake, and no estimate of variability was available for several of the other mushrooms so it was not possible to make rigorous statistical comparisons. Compared to previous data in SR, the assayed total folate content determined by LC–MS in the present study was generally higher in the crimini and uv-treated portabella, and markedly lower in the maitake mushrooms. The 2009 values in SR for enoki, maitake, oyster, portabella (non-uv-treated), and shiitake mushrooms were obtained from an earlier 2005 nationwide sampling and data for white mushrooms were based on the analysis of the 2005 samples and as well as literature sources. Total folate was determined at a qualified commercial laboratory using standard microbiological methodology (Angyal, 1996), and many of the values were based on the analysis of only one or two composites, resulting in no standard error reported in SR. Compared to total folate concentrations reported by Mattila et al. (2001) that were also determined by microbiological assay, the present study showed much lower values in white button and shiitake mushrooms. Due to the documented high variability in the microbiological assay (Koontz et al., 2005) and the correspondingly limited analyses on which the microbiologically determined means from these other studies are based, it is impossible to evaluate precise reasons for the differences in observed concentrations.

This study confirms mushrooms as a good source of folate and provides detailed and chemically specific data on the folate composition of several types of mushrooms. Enoki and oyster mushrooms appear to be superior sources of folate compared to the *Agaricus bisporus* varieties (white button, portabella, crimini) that are more commonly consumed in the U.S., and also relative to maitake and shiitake. Morel and chanterelle mushrooms were exceptions in being relatively poor sources of folate, containing only 2.22–6.40 µg/100 g. Excluding these two, based on the average assayed folate concentration for each product and assuming a serving size of 1 cup sliced mushrooms (~70 g), the dietary folate contribution per serving would be 9 µg (shiitake) to 30–31 µg (enoki and oyster), representing 2% to 8% of the RDA of 400 µg for individuals age 14 and older in the U.S. (National Academy of Sciences, 1998). The high concentration of naturally occurring folates in mushrooms makes them a good source of this vitamin for individuals with MTHFR mutations (Gilbody et al., 2007), and also in diets that lack folic acid enriched foods.

Food composition data for naturally occurring folates in addition to total folate are useful for dietary recommendations and for research on the impact of dietary folate and natural folates on health and disease. Selecting mushroom types that are higher in folate (e.g., oyster, enoki) could enhance natural folate intake. It should be noted that the sampling plan for this study was designed to obtain products representative of those in the U.S. marketplace. Generalisation of these data to samples of these mushrooms and other species that are consumed around the world should be made only with the recognition that folate content could be affected by growing conditions and possibly other factors, and therefore the values may not accurately represent other food supplies. In particular, morel mushrooms comprise a wide range of species, so the present study would certainly not capture all possible variability. Similarly, estimating folate intake from consumption of specific samples (e.g., in feeding trials) using average values for the products that showed high sample-to-sample variability in the U.S. samples could result in a large deviation between the estimated and actual intake. For example, the highly consumed white button and portabella mushrooms had the narrowest difference (2.83–6.01 µg/100 g) in folate content among separate composites, suggesting that average food composition values would provide a reliable estimate for any individual sample of these products. However, for other types of mushrooms showing high variability it could be expected that the folate content of a particular sample might differ significantly from the average. For example, crimini, enoki, and oyster mushrooms had differences of 25.5–47.3 µg/100 g among the composites with the highest and lowest folate within each species.

Finally, it is extremely important that appropriate method validation and quality control measures are included in any studies of biodiversity in folate composition, to eliminate analytical variability and error from any actual sample to sample differences.

Folate data from the current study were included in an update to SR (http://www.ars.usda.gov/Services/docs.htm?docid=8964) in 2010. Sterols and vitamin D were also analysed in the same mushroom samples, and those results will be reported in a separate publication.

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References

Angyal, G. (1996). *U.S Food and Drug Administration methods for the microbiological analysis of selected nutrients*. Gaithersburg, MD: AOAC International.

Ashfield-Watt, P. A., Whiting, J. M., Clark, Z. E., Moat, S. J., Newcombe, R. G., Burr, M. L., et al. (2003). A comparison of the effect of advice to eat either ''5-a-day'' fruit and vegetables or folic acid-fortified foods on plasma folate and homocysteine. *European Journal of Clinical Nutrition, 57*, 316–323.

Cağlarırmak, N. (2007). The nutrients of exotic mushrooms (*Lentinula edodes* and *Pleurotus* species) and an estimated approach to the volatile compounds. *Food Chemistry, 105*, 1188–1194.

Cossins, E. A., & Liangfu, C. (1997). Folates and one-carbon metabolism in plants and fungi. *Phytochemistry, 45*, 437–452.

DeVries, J. W., Keagy, P. M., Hudson, C. A., & Rader, J. I. (2001). AACC collaborative study of a method for determining total folate in cereal products by microbiological assay using trienzyme extraction (AACC method 86–47). *Cereal Foods World, 46*, 216–219.

DeVries, J. W., Rader, J. I., Keagy, P. M., Hudson, C. A., Angyal, G., Arcot, J., et al. (2005). Microbiological assay-trienzyme procedure for total folates in cereals and cereal foods: collaborative study. *Journal of the Association of Official Analytical Chemists International, 88*, 5–15.

Finglas, P. M., Scott, K. J., Witthöft, C. M., van den Berg, H., & de Froidmont-Görtz, I. (1998). The certification of the mass fractions of vitamins in four reference materials: wholemeal flour (CRM 121), milk powder (CRM 421), lyophilised mixed vegetables (CRM 485), and lyophilised pigs liver (CRM 487). EUR Report 18320. Luxembourg: Office for Official Publications of the European Communities.

Finglas, P. M., Wigertz, K., Vahteristo, L., Witthöft, C., Southon, S., & de Froidmont-Görtz, I. (1999). Standardisation of HPLC techniques for the determination of naturally-occurring folates in food. *Food Chemistry, 64*, 245–255.

Freisleben, A., Schieberle, P., & Rychlik, M. (2003). Comparison of folate quantification in foods by high-performance liquid chromatography–fluorescence detection to that by stable isotope dilution assays using high-performance liquid chromatography–tandem mass spectrometry. *Analytical Biochemistry, 315*, 247–255.

Gilbody, S., Lewis, S., & Lightfoot, T. (2007). Methylenetetrahydrofolate reductase (MTHFR) genetic polymorphisms and psychiatric disorders: A HuGE review. *American Journal of Epidemiology, 165*, 1–13.

Haytowitz, D. B., Pehrsson, P. R., & Holden, J. M. (2007). The National Food and Analysis Program: A decade of progress. *Journal of Food Composition and Analysis, 21*(Supp. 1), S94–S102. doi:10.1016/j.jfca.2007.07.006.

Horwitz, W., Kamps, L. R., & Boyer, K. W. (1980). Quality assurance in the analysis of foods for trace constituents. *Journal of the Association of Official Analytical Chemists, 63*, 1344–1354.

Kalač, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: a review. *Food Chemistry, 113*, 9–16.

Kariluoto, M. S., Vahteristo, L. T., & Piironen, V. I. (2001). Applicability of microbiological assay and affinity chromatography purification followed by high-performance liquid chromatography (HPLC) in studying folate contents in rye. *Journal of the Science of Food and Agriculture, 81*, 938–942.

Kim, Y.-I. (2003). Role of folate in colon cancer development and progression. *Journal of Nutrition, 133*, 731S–3739S.

Konings, E. J. M. (1999). A validated LC method for the determination of folates in vegetables, milk powder, liver and flour. *Journal of the Association of Analytical Chemists International, 82*, 119–127.

Konings, E. J. M. (2006). General Referee Report, Committee on Food Nutrition: Water-soluble vitamins. *Journal of the Association of Official Analytical Chemists International, 89*, 1–5.

Konings, E. J. M., Roomans, H. H., Dorant, E., Goldbohm, R. A., Saris, W. H., & van den Brandt, P. A. (2001). Folate intake of the Dutch population according to newly established liquid chromatography data for foods. *American Journal of Clinical Nutrition, 73*, 765–776.

Koontz, J. L., Phillips, K. M., Wunderlich, K. M., Exler, J., Holden, J. M., Gebhardt, S. E., et al. (2005). Comparison of total folate concentration in foods determined by microbiological assay at several experienced laboratories. *Journal of the Association of Official Analytical Chemists International, 88*, 805–813.

Last, A. R., & Wilson, S. A. (2006). Low-carbohydrate diets. *American Family Physician, 73*, 1942–1948.

Lucock, M. (2000). Folic acid: Nutritional biochemistry, molecular biology, and role in disease processes. *Molecular Genetics and Metabolism, 71*, 121–138.

Mattila, P., Könkö, K., Eurola, M., Pihlava, J.-M., Astola, J., Vahteristo, L., et al. (2001). Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. *Journal of Agricultural and Food Chemistry, 49*, 2343–2348.

Moat, S. J., Lang, D., McDowell, I. F. W., Clarke, Z. L., Madhavan, A. K., Lewis, M. J., et al. (2004). Folate, homocysteine, endothelial function and cardiovascular disease. *Journal of Nutritional Biochemistry, 15*, 64–79.

Muskiet, F. A., & Kemperman, R. F. (2006). Folate and long-chain polyunsaturated fatty acids in psychiatric disease. *Journal of Nutritional Biochemistry, 17*(11), 717–727.

National Academy of Sciences, Institute of Medicine. (1998). *Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline*. Washington, DC: National Academy Press. Available from http://darwin.nap.edu/books/0309065542/html/196.html.

Patring, J., Wandel, M., Jägerstad, M., & Frølich, W. (2009). Folate content of Norwegian and Swedish flours and bread analysed by use of liquid chromatography–mass spectrometry. *Journal of Food Composition and Analysis, 22*, 649–656.

Pehrsson, P., Haytowitz, D., Holden, J., Perry, C., & Beckler, D. (2000). USDA's National Food and Nutrient Analysis Program: Food sampling. *Journal of Food Composition and Analysis, 13*, 379–389.

Phillips, K. M., Patterson, K. Y., Rasor, A. R., Exler, J., Haytowitz, D. M., Holden, J. M., et al. (2006). The role of quality control and reference materials in the National Food and Nutrient Analysis Program. *Analytical and Bioanalytical Chemistry, 384*, 1341–1355.

Phillips, K. M., Ruggio, D. M., Ashraf-Khorassani, M., Eitenmiller, R. R., Cho, S., Lemar, L. E., et al. (2010). Folic acid content of ready-to-eat cereals determined by liquid chromatography-mass spectrometry: Comparison to product label and to values determined by microbiological assay. *Cereal Chemistry, 87*, 42–49. doi:10.1094/CCHEM-87-1-0042.

Phillips, K. M., Ruggio, D. M., Ashraf-Khorassani, M., & Haytowitz, D. B. (2006). Difference in folate content of green and red sweet peppers (*Capsicum annuum*) determined by liquid chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry, 54*, 9998–10002.

Phillips, K. M., Wunderlich, K. M., Exler, J., Holden, J. M., Gebhardt, S. E., Haytowitz, D. B., et al. (2005). Stability of 5-methyltetrahydrofolate in fresh frozen fruits and vegetables. *Food Chemistry, 94*, 587–595.

Puwastien, P., Pinprapaia, N., Judprasonga, K., & Tamura, T. (2005). International inter-laboratory analyses of food folate. *Journal of Food Composition and Analysis, 18*, 387–397.

Quinlivan, E. P., Hanson, A. D., & Gregory, J. F. (2006). The analysis of folate and its metabolic precursors in biological samples. *Analytical Biochemistry, 348*, 163–184. doi:10.1016/j.ab.2005.09.017.

Regland, B. (2005). Schizophrenia and single-carbon metabolism. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 29*, 1124–1132.

Robien, K., & Ulrich, C. M. (2003). 5,10-Methylenetetrahydrofolate reductase polymorphisms and leukemia risk: A HuGE minireview. *American Journal of Epidemiology, 157*, 571–582.

Rychlik, M. (2004). Revised folate content of foods determined by stable isotope dilution assays. *Journal of Food Composition and Analysis, 17*, 475–483.

Rychlik, M., Englert, K., Kapfer, S., & Kirchhoff, E. (2007). Folate contents of legumes determined by optimized enzyme treatment and stable isotope dilution assays. *Journal of Food Composition and Analysis, 20*, 411–419.

Sharp, L., & Little, J. (2004). Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: A HuGE review. *American Journal of Epidemiology, 159*, 423–443.

Storozhenko, S., Ravanel, S., Zhan, G.-F., Rébeillé, F., Lambert, W., & Van Der Straeten, D. (2005). Folate enhancement in staple crops by metabolic engineering. *Trends in Food Science and Technology, 16*, 271–281.

Stover, P. J. (2004). Physiology of folate and vitamin B12 in health and disease. *Nutrition Reviews, 62*, S3–S12.

U.S. Food and Drug Administration. (1996). Food Standards: Amendment of standards of identity for enriched grain products to require addition of folic acid. *Final Rule. 61 Federal Register 44 (5 March 1996)*, (pp. 8781–8797). Available from http://vm.cfsan.fda.gov/~lrd/fr96305b.html.

USDA Agricultural Research Service. (2009). USDA National Nutrient Database for Standard Reference, Release 22. URL: http://www.ars.usda.gov/nutrientdata (accessed August 2009).

Yon, M., & Hyun, T. H. (2003). Folate content of foods commonly consumed in Korea measured after trienzyme extraction. *Nutrition Research, 23*, 735–746.